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STOCKHOLM,
August 24, 1910

SPECIAL ARTICLES

THE PERMEABILITY AND CYTOLYSIS OF EGGS

THE question as to whether cells may change in their permeability to various substances and the bearing of these changes on vital phenomena, in particular the development of the egg, has excited much discussion of late. My own experiments (at Tortugas and Woods Hole) have been directed toward determining the permeability of sea-urchin's eggs for some one substance and the effect of certain substances in altering the permeability of the egg for this substance. Sodium hydrate was chosen for the purpose because its entrance may readily be indicated after staining the cells in neutral red. This dye, red in neutral and acid solution, becomes yellow in alkaline solution.

Since the classic researches of Pfeffer it has been well known that plant cells will take up and concentrate in their sap vacuoles, certain dyes, notably methylene blue, from very dilute solutions. In some cells the dye is precipitated as fine blue granules which Pfeffer proved to be a compound formed with tannic acid. In the leaf cells of *Elodea* the dye remains in solution yet becomes more concentrated than without. This gives the appearance of a diffusion of the dye into the cell against a concentration gradient, which is of course an impossibility. The dye must be changed within, but "the precise character of the still soluble combination in which pigment accumulates in the cell sap of *Trianea, Lemna, Elodea,* etc., is as yet unknown."[1] A clue as to the nature of the condition in which the dye exists in the cell is obtained by staining with neutral red. In tap water a dilute solution is brick red, indicating that a small amount of the dye is present in the alkaline yellow condition, the undissociated molecule (ROH), on the theory of indicators. But in the *Elodea* cells it is always bright red in color. This suggested that within the cells the neutral red existed in the acid or dissociated (RCl) condition which was unable to pass out. That this view is correct can be easily shown by placing *Elodea* leaves in tap water containing neutral red plus just enough acid to convert all the dye into the acid condition without injuring the cells themselves. Not a cell stains. Thus the protoplasts "select" only the undissociated molecules of basic dyes from a solution. Within the cell these are practically completely dissociated and unable to pass out, giving the appearance of diffusion against a concentration gradient. Exactly the same conditions hold for methylene blue, only there is no difference in the color of the dissociated and undissociated elements.

Sea-urchin eggs are also capable of concentrating neutral red from very dilute solutions, but the manner of retaining the dye is very different, although the conditions of entrance are the same. No neutral red can enter sea-urchin eggs from dilute acid sea water in concentrations which do not coagulate the egg. As soon as the eggs do coagulate they stain but in a different manner from the normal eggs. In the latter the dye is taken up (combined?) by granules distinguishable from the yolk granules, in that they always pass to the distal pole of the egg on centrifuging, whether first stained and then centrifuged or first centrifuged and then stained. They are present in the fertilized as well as the unfertilized egg (*Toxopneustes*), but the rate of staining of these granules is much more rapid in the former than the latter. I would attribute this to the rate of entrance of the dye and can therefore confirm for neutral red what Lyon has recently found for methylene blue.

If red-stained sea-urchin eggs are placed in hyperalkaline (100 c.c. sea water + 1.3 c.c. $n/10$ NaOH) sea water they retain their red color for several hours. When killed by chloroform-saturated sea water, the alkali almost instantly enters and turns the red to yellow. It may be shown that the color change is independent of the swelling of the egg caused by chloroform, for the penetration

[1] Pfeffer's "Plant Physiology," Vol. I., p. 94, 1900.

of alkali takes place just the same when swelling is prevented (for a short time) by the addition of cane sugar to the sea water. Either the NaOH fails to enter normal eggs or only enters so slowly that it is neutralized within the egg. Since the number of eggs is very small compared with the bulk of alkaline solution, and the alkali would continue to diffuse in, so long as neutralized, it would require an enormous production of acid on the part of the egg to take care of the NaOH entering. For this reason the first alternative seems more probable.

By treating eggs stained in neutral red with stronger and stronger concentrations of NaOH in 0.6 *m* NaCl the alkali penetrates more and more rapidly. If a concentration of NaOH which enters the egg in twenty minutes be one quarter saturated with chloroform, the NaOH enters in ten minutes. One quarter saturated chloroform in NaCl has no visible effect on the eggs even after one hour. The effect of dilute solutions of chloroform, which *fail to cytolyze,* on the eggs of *Hipponoë,* is to increase their permeability to NaOH. Indeed it may be shown in the same way, that small concentrations of chloroform increase the permeability of the leaf cells of *Elodea,* showing active protoplasmic rotation, and that the normal impermeability is again regained when the leaves are returned to tap water. The above statements are equally true for ether. It is obvious that the number of substances whose effect on the cell surface we may test in this way is limited, for most of them combine with NaOH.

Thus one of the most effective substances for producing artificial membranes, chloroform, increases the permeability of the egg for alkali. By comparing the time it takes for the stained eggs, fertilized and unfertilized, to change from red to yellow, in the same solution of NaOH, it is found that just after fertilization (2–5 minutes) the egg is much more permeable to NaOH, despite the fact that it is surrounded by a fertilization membrane. While my experiments were being performed I was unaware that Loeb had been studying the relative injurious or destructive action of alkali on the fertilized and unfertilized eggs and had found the former to be most quickly injured. My results with red-stained eggs show that the injurious action is actually due to the penetration of alkali. The same is true if we form the membrane artificially by treatment with acetic acid (in *Asterias*). In the only series of experiments performed, with *Toxopneustes* eggs, between ten and fifteen minutes after fertilization the eggs return to the same condition of permeability, with respect to alkali, as the unfertilized. There appears to be a second increase at the time of first cleavage.

Thus far I have found no dye which is harmless for eggs and at the same time changes in color in acids. The egg of *Hydractinia,* however, contains a natural green pigment which becomes red in HCl but not green again in alkali. The color change also takes place when the eggs are slowly heated.

If we place unfertilized *Hydractinia* eggs in 50 c.c. sea water $+ 3$ c.c. $n/10$ HCl it takes half an hour for the green pigment to become red. If first treated with chloroform-saturated sea water, and then placed in the acid sea water the color change is almost instantaneous. Thus the normal eggs are relatively impermeable to HCl. Unfortunately carbonic acid is too weak to affect the color of the green pigment, and we can not test the permeability of fertilized and unfertilized eggs to CO_2. The end of the breeding season has made a comparison of the entrance of HCl into the fertilized and unfertilized eggs impossible.

A phenomenon that occurs, if the treatment of an egg with chloroform and many other membrane-forming substances be prolonged, is cytolysis. It is characterized by the swelling of the egg and the decomposition of the visible granules which appear to fuse to larger more liquid spheres, with a loss of their natural pigment or of their stain if first placed in a solution of neutral red. By the use of the centrifuge, which brings about such a distribution of the chemical constituents of the egg that we can readily see exactly what happens to each of them, it may

be shown that only the yolk and pigment granules break down. The oil is unaffected. The eggs are first centrifuged and then cytolyzed (with $CHCl_3$ or saponin) or first cytolyzed and then centrifuged. Swelling and break down of the granules take place simultaneously and suddenly. It is impossible to say which precedes and which follows. Many eggs contain granules which do not break down on cytolysis although the whole egg swells so that it would appear as if the connection of the granules in an egg with cytolysis were purely secondary and that chloroform or saponin does not combine with them and break them up. Clear fragments of eggs cytolyze as readily as granular fragments.

This supposition is further supported by the fact that the granules (excepting oil) of *Arbacia* eggs are broken up into *exactly the same products characteristic of cytolysis when the eggs are crushed in sea water.* There must be something in the egg in whose presence the granules are stable or something in the sea water in whose presence they are unstable, and the egg surface forms an impenetrable barrier for this substance. The latter alternative may be tested much more easily experimentally and it appears probable that the calcium salts of sea water are chiefly responsible for the breakdown of the yolk and pigment granules. If crushed in pure 0.6 *m* NaCl or KCl the granules retain their color and integrity. If cytolyzed in pure NaCl the granules remain intact, although they are changed in some way, for any dye or pigment they may contain passes out of them. The whole egg nevertheless swells. The cytolysis of the sea-urchin egg would be in all respects like that of the annelid were it not for the calcium of the sea water. The yolk granules of annelid eggs are stable in sea water.

The relation of calcium to cytolysis shows further that it is not present in the egg in the same condition as in the sea water and does not pass into the egg in that condition unless its surface has been destroyed by some cytolytic substance.

The conception of cytolysis to which I have been led is essentially that of Hamburger, Koeppe and other physiologists. It is certain that during cytolysis there is a progressive change from impermeability to complete permeability for most diffusible substances, for it is a change from a definite plasma membrane to no true surface whatsoever, if the eggs remain in the solution long enough. The apparent surface of cytolyzed eggs is merely the artificial fertilization membrane formed in the first stages of cytolysis. The permeability of the plasma membrane is increased to a certain extent and under these conditions substances pass out which form an albuminoid membrane. In the next stage the egg becomes permeable to the salts of sea water when swelling of the egg and disintegration of the granules takes place, and at the same time the egg surface loses its continuity.

The cause of the swelling is simply the substitution of a surface freely permeable to salts for one quite impermeable to them. In the normal egg the sum of the osmotic pressures of the substances within the egg just counterbalances that of the salts of sea water. This does not mean that the substances within the egg are the same as those without. The egg is not a mass of proteid saturated with sea water, but even its salt content is different. Suppose the membrane separating these two phases becomes permeable for the dissolved substances of one phase but not for those of the other. The result is the same as placing the cell in distilled water. It swells until its turgor pressure is balanced by the tension of its artificial membrane.

The slowness with which eggs swell when placed in distilled water, considering the large surface area, points to the view that even water encounters resistance in its exit from and entrance into the egg. In distilled water the egg slowly swells to a certain size, at which point it suddenly swells and a delicate membrane forms. I am inclined to believe that at this point the surface becomes freely permeable to the entrance of water and the whole egg swells (within the artificial

membrane which forms at the same time) until its internal pressure is compensated by the tension of its membrane.

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August 31, 1910

COMPARATIVE ANALYSES OF WATER FROM THE GREAT SALT LAKE

From about 1900 until 1904 fears were expressed that the Great Salt Lake was doomed to extinction, and that it would be a matter of only a few years until its site would become a salt desert. The recession of the shore line and sinking of the lake level continued until the autumn of 1903. Since that time there has been a rise in the level of the lake, and during the year just ending new fears have arisen—fears that large engineering works like the Lucin cut-off of the Southern Pacific and the roadbed of the Western Pacific railroad would have to be abandoned. A succession of years with abnormally high rainfall is responsible for the condition now existing.

TABLE I

Date of Collection	Specific Gravity	Total Solids Per Cent. by Weights	Grams Liter	Authority
1850	1.170	22.282	260.69	L. D. Gale
Summer 1869	1.111	14.9934	166.57	O. D. Allen
August 1873	1.102	13.42	147.88	H. Bassett
December 1885	1.1225	16.7162	187.65	J. E. Talmage
February 1888	1.1261			J. E. Talmage
June 1889	1.148			J. E. Talmage
August 1889	1.1569	19.5576	226.263	J. E. Talmage
August 1892	1.156	20.51	238.12	E. Walker
September 1892	1.1679	21.47	250.75	J. E. Talmage
1893		20.05		J. T. Kingsbury
December 1894	1.1538	21.16	244.144	J. E. Talmage
May 1895	1.1583	21.39	247.760	J. E. Talmage
June 1900	1.1576	20.90	241.98	H. N. McCoy and Thomas Hadley
July 1900	1.1711	22.89	268.09	H. W. Sheley
August 1900	1.1805	23.36	275.765	H. W. Sheley
October 1900	1.1860	24.03	285.020	H. W. Sheley
September 1901	1.1979	25.221	302.122	L. J. Seckles
October 1903	1.2206	27.72	338.36	William Blum
June 1904	1.1905	25.196	299.96	J. E. Talmage
November 1904	1.2120	26.71	323.71	William Blum
October 1907	1.1810	22.92	270.685	W. C. Ebaugh and Kenneth Williams
October 1909	1.1561	20.887	242.25	Wallace Macfarlane
February 1910	1.1331	17.681	200.32	Wallace Macfarlane

The above values are taken in part from "The Great Salt Lake," by J. E. Talmage, and all the analyses during recent years have been made in the laboratories of the University of Utah.

TABLE II

Sample Collected	Oct., 1903	Nov., 1904	Oct., 1907	Oct., 1909	Feb., 1910
Specific gravity	1.2206	1.2120	1.1810	1.1561	1.1331
Total solids	27.72 %	26.71 %	22.92 %	20.88 %	17.68 %
Constituents					
Chlorine (Cl)	15.27 %	14.54 %	12.67 %	10.91 %	9.48 %
Sulphate (SO_4)	1.86	1.82	1.53	1.39	1.05
Magnesium (Mg)	0.155	0.43	0.45	0.447	0.391
Calcium (Ca)	0.045	0.055	0.04	0.080	0.055
Sodium (Na)	9.58	8.77	7.58	7.25	5.79
Potassium (K)	0.73	0.89	0.72	0.76	0.88

An inspection of the results of analyses of the lake water will be of interest. In Table I. are shown the specific gravity and total solids obtained by investigators at various times during the last forty or more years, and in Table II. more complete results of the latest analyses are recorded. In this connection, it should be remembered that the annual variation of the lake water shows a minimum of total solids in the spring, following the winter and spring precipitation, and a maximum in the autumn.

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A RARE FISH FROM THE NEW JERSEY COAST

A specimen of *Polyprion americanus* (Bloch and Schneider) was captured with hook and line by Captain Harry Maddox, eight miles off Asbury Park, N. J., on August 21, 1910. This species, known as the wreck-fish or stone-bass is said to be not uncommon in European waters, where it reaches a large size—five to six feet in length. Only a single specimen has been recorded heretofore on the American side of the Atlantic, taken by the U. S. Fish Commission in the Gulf Stream off the Grand Banks.

The specimen taken by Captain Maddox is therefore not only new to the New Jersey list, but is also the first to be recorded near the coast of the United States. It measured a trifle over ten inches and weighed thirteen ounces.

It was sent to the New York Aquarium for identification and has been turned over to the collection of the American Museum of Natural History.

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The New York Aquarium